RIPK2 Kinase Assay

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Scientific Background:

RIPK2 (RIP2; RICK) is a death domain-containing protein kinase encoding a predicted 540-amino acid protein which contains an N-terminal serine/threonine kinase catalytic domain and a C-terminal caspase activation and recruitment domain. RIPK2 is thought to regulate apoptosis induced by the FAS receptor pathway (1). RIPK2 has been shown to specifically interact with the CARD of ICE/caspase-1 and this interaction correlates with the processing of pro-caspase-1 and the formation of the active caspase-1 p20 (2).


ADP-Glo™ Kinase Assay

Description
ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase (Fig. 1). The luminescent signal positively correlates with ADP amount (Fig. 2) and kinase activity (Fig. 3A). The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling (Fig. 3B). The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.
Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1 μl of inhibitor or (5% DMSO)
  - 2 μl of enzyme (defined from table 1)
  - 2 μl of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5 μl of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 μl of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1 second).

Table 1. RIPK2 Enzyme Titration. Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

<table>
<thead>
<tr>
<th>RIPK2, ng</th>
<th>200</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>12</th>
<th>6.3</th>
<th>3.1</th>
<th>1.6</th>
<th>0.8</th>
<th>0</th>
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<tbody>
<tr>
<td>RLU</td>
<td>170705</td>
<td>93670</td>
<td>57115</td>
<td>31430</td>
<td>16638</td>
<td>8747</td>
<td>4740</td>
<td>2649</td>
<td>1718</td>
<td>909</td>
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<tr>
<td>S/B</td>
<td>187.8</td>
<td>103.0</td>
<td>62.8</td>
<td>34.6</td>
<td>18.3</td>
<td>9.6</td>
<td>5.2</td>
<td>2.9</td>
<td>1.9</td>
<td>1</td>
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<tr>
<td>% Conversion</td>
<td>59.8</td>
<td>32.6</td>
<td>19.7</td>
<td>10.7</td>
<td>5.5</td>
<td>2.7</td>
<td>1.3</td>
<td>0.5</td>
<td>0.2</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 3. RIPK2 Kinase Assay Development. (A) RIPK2 enzyme was titrated using 50μM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 10ng of RIPK2 to determine the potency of the inhibitor (IC50).

Assay Components and Ordering Information:

<table>
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<tr>
<th>Products</th>
<th>Company</th>
<th>Cat.#</th>
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<tr>
<td>ADP-Glo™ Kinase Assay</td>
<td>Promega</td>
<td>V9101</td>
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<tr>
<td>RIPK2 Kinase Enzyme System</td>
<td>Promega</td>
<td>V4084</td>
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<tr>
<td>ADP-Glo™ + RIPK2 Kinase Enzyme System</td>
<td>Promega</td>
<td>V4085</td>
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RIPK2 Kinase Buffer: 40mM Tris, 7.5; 20mM MgCl2; 0.1mg/ml BSA; 50μM DTT.